Research article

LUMINESCENCE
The Journal of Biological and Chemical Luminescence

Received: 13 July 2014, Revised: 12 October 2014, Accepted: 24 October 2014 Published online in Wiley Online Library: 16 December 2014

(wileyonlinelibrary.com) DOI 10.1002/bio.2825

Long-term chemiluminescence signal is produced in the course of luminol oxidation catalyzed by enhancer-independent peroxidase purified from *Jatropha curcas* leaves

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ABSTRACT: Isoenzyme *c* of horseradish peroxidase (HRP-C) is widely used in enzyme immunoassay combined with chemiluminescence (CL) detection. For this application, HRP-C activity measurement is usually based on luminol oxidation in the presence of hydrogen peroxide (H_2O_2). However, this catalysis reaction was enhancer dependent. In this study, we demonstrated that *Jatropha curcas* peroxidase (JcGP1) showed high efficiency in catalyzing luminol oxidation in the presence of H_2O_2. Compared with HRP-C, the JcGP1-induced reaction was enhancer independent, which made the enzyme-linked immunosorbent assay (ELISA) simpler. In addition, the JcGP1 catalyzed reaction showed a long-term stable CL signal. We optimized the conditions for JcGP1 catalysis and determined the favorable conditions as follows: 50 mM Tris buffer (pH 8.2) containing 10 mM H_2O_2, 14 mM luminol and 0.75 M NaCl. The optimum catalysis temperature was 30°C. The detection limit of JcGP1 under optimum condition was 0.2 pM. Long-term stable CL signal combined with enhancer-independent property indicated that JcGP1 might be a valuable candidate peroxidase for clinical diagnosis and enzyme immunoassay with CL detection. Copyright © 2014 John Wiley & Sons, Ltd.

Keywords: peroxidase; chemiluminescence; luminol; *Jatropha curcas*

Introduction

Class III plant peroxidase (EC 1.11.1.7) is one of the most widely known plant enzymes. This enzyme plays a role in diverse physiological processes (1). The peroxidases have been isolated, purified and characterized from various plant sources and are broadly used as a model enzyme to investigate enzyme structures and properties (2). However, only the cationic isoenzyme *c* from horseradish peroxidase (HRP-C) has been developed thoroughly for practical applications (3–6). HRP-C has been widely applied as an enzyme label by conjugation with antibody or antigen in enzyme-linked immunosorbent assay (ELISA) (7). To detect enzymatic activity, chemiluminescence (CL) is commonly used because the CL method is more sensitive when compared with colorimetry or fluorimetry (8,9). In the CL method, the measurement of HRP-C activity is usually based on oxidation of luminol in the presence of H_2O_2.

Certain enhancers are essential for the HRP-C CL reaction to obtain a higher CL signal (10). The mechanism of the enhanced CL reaction is different with that of the non-enhanced CL reaction (11,12). In brief, the enhancer (AH), instead of luminol, is first oxidized by H_2O_2 in the presence of HRP-C because the enhancer is more active towards HRP-C than luminol in the reaction solutions (eqns (1)–(3)), known as the 'ping-pong' mechanism. These reactions form the radical product of a one-electron oxidation enhancer (A•), which can oxidize luminol. Eventually luminol is converted into 3-aminophthalate, and this process results in light emission (eqn (4)). In these equations, E represents the resting-state peroxidase while EI and EII represent its intermediate compounds:

$$E + H_2O_2 \rightarrow EI + H_2O \quad (1)$$

$$EI + AH \rightarrow EII + A\bullet \quad (2)$$

$$EII + AH \rightarrow E + A\bullet + H_2O \quad (3)$$

$$A\bullet + \text{luminol} \rightarrow AH + 3-\text{aminophthalate} + N_2 + h\upsilon \quad (4)$$

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Abbreviations: ELISA, enzyme-linked immunosorbent assay; LDL, lower detection limit; RLU, relative luminescence units; SPP, sweet potato peroxidase.

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The absence of enhancer could slow down light emission in the luminol oxidation reaction, so several enhancers have been developed (12). The enhancer *p*-iodophenol was been added to the substrate mixture, but only low stability of CL signal was obtained in the *p*-iodophenol-enhanced CL reaction catalyzed by HRP-C, which might lead to an inaccurate estimation of analytes (13). Other cocktails of enhancers have been developed to improve the signal stability and sensitivity of this reaction (14) but, for practical purposes, a simpler reaction mixture might have been better.

Recently, some studies have investigated that oxidation of luminol could be catalyzed by several anionic peroxidases, e.g. soybean peroxidase (SBP) (15), African oil palm tree peroxidase (AOPTP) (16), sweet potato peroxidase (SPP) (17) and royal palm leaf peroxidase (RPLP) (18). The catalysis mechanism of these anionic peroxidases is similar for HRP-C except for their capability to catalyze luminol oxidation without an enhancer. For these anionic peroxidases, AH and A• in the above equations represent luminol and its radical product from one-electron oxidation. The catalysis reaction by these anionic peroxidases showed a long-term stable CL signal (15–18). In addition, some of these enzymes have been successfully developed for sensitive ELISA assay (19,20).

Here we demonstrate that *Jatropha curcas* peroxidase (JcGP1), a novel highly stable anionic enzyme isolated from *Jatropha curcas*, was able to catalyze luminol oxidation without any enhancer. The optimal reaction conditions, the kinetic curve, as well as the lower detection limit of the JcGP1 induced CL reaction were analyzed in this study.

Experimental

Reagents

Jatropha curcas peroxidase (JcGP1, RZ 3.2) was purified from leaves as described previously (21). Horseradish peroxidase (HRP-C, RZ 3.0) and soybean peroxidase (SBP, RZ 3.2) were purchased from Sigma (USA) and Bio-Research Products (USA), respectively. Luminol, Tris, *p*-iodophenol and H_2O_2 (30%) were obtained from Sigma (USA). The concentrations of HRP-C and SBP were measured using $\varepsilon_{402} = 102\ 000\ M^{-1}\ cm^{-1}$ and $\varepsilon_{403} = 90\ 000\ M^{-1}\ cm^{-1}$, respectively (22,23). The concentration of JcGP1 was determined by spectrophotometry according to haem absorbance $\varepsilon_{403} = 102\ 000\ M^{-1}\ cm^{-1}$ (13). The concentration of H_2O_2 was measured by $\varepsilon_{240} = 43.6\ M^{-1}\ cm^{-1}$ (24). Dilutions of H_2O_2 were freshly prepared.

Catalytic luminol oxidation conditions

The catalysis for luminol oxidation was performed as follows: 240 μL of Tris–HCl buffer (20–200 mM, pH 7.6–9.4) containing H_2O_2 (2–20 mM), luminol (0.1–20 mM) and NaCl (0–2.5 M) were introduced into the well of a white polystyrene plates (MaxiSorp, Nunc, Denmark). Then, the enzymatic reaction was initiated by adding 10 μL of peroxidase solution. The CL kinetics was recorded for 180 min at various temperatures (25–45°C) on a luminometer (Bio-Tek, USA). The CL intensity in the absence of enzyme was measured as the background. The CL intensity of peroxidase, defined as relative luminescence units (RLU) was obtained by subtracting the CL intensity of background from the total CL intensity. Experiments were performed in triplicate.

Evaluation of the CL kinetics properties

In order to optimize the reaction condition, three parameters were defined to evaluate the CL kinetics properties. MI represents the maximum intensity of CL in each condition. DMI represents the duration of MI when the CL intensity reaches a plateau above 98% of maximum CL intensity. The requirement of DMI was set as no shorter than 20 min. TTMI represents the time from initiation of the reaction to the MI. The requirement of TTMI was set as between 7–15 min. To determine the favorable conditions for each parameter, the requirements of the DMI and TTMI were first considered. Next, within the range determined by DMI and TTMI, the maximal MI was chosen. Data were collected and analyzed in each optimization trial.

Results and discussion

Anionic peroxidase from *Jatropha curcas* leaves (JcGP1) was purified first by Feng in 2012. This enzyme is a glycoprotein with a molecular mass of 48 000 Da. It belongs to the family of secretory plant peroxidases (21). Isoelectric focusing showed that the pI of JcGP1 was 3.89, indicating that it was an anionic peroxidase (data not shown).

Optimization of catalytic luminol oxidation

Our goal was to obtain a lower detection limit and a stable CL signal, which are critical for practical application. First, we optimized the parameters of the JcGP1-catalyzed luminol reaction. Note that the standards to determine the optimal conditions may vary due to different practical purposes. In the study, considering the high sensitivity requirement and time frame for setting up a multi-well plate diagnostic kit (typically 10–20 min), the requirements of DMI and TTMI were set as described above.

As shown in Fig. 1(A), MI, DMI and TTMI were all strongly affected by pH of the reaction mixture. The optimum pH for MI was 8.1–8.3, which was close to that of HRP-C (pH 8.3) (14), SBP (pH 8.4–8.6) (15), AOPTP (pH 8.3–8.6) (16) and RPLP (pH 8.3–8.6) (18). However, to meet the requirements of DMI and TTMI, the pH needs to be no less than 8.2. Therefore, pH 8.2 was determined as the optimum pH for the catalysis reaction to obtain a higher MI.

Previous literature has indicated that JcGP1 exhibits hot preference. The maximum activity of JcGP1 was found at 60°C using guaiacol as substrate (21). Therefore, it was necessary to investigate the effect of reaction temperature on JcGP1 catalytic activity. As shown in Fig. 1(B), the phenomenon of hot activation was also observed in the luminol oxidation catalysis. In contrast, DMI and TTMI were negatively correlated with reaction temperature. According to our optimization criteria, we considered that the optimum temperature for the catalysis reaction was 30°C.

H_2O_2 is an oxidative substrate for peroxidases and produces compound I from a resting form of peroxidase (eqn (1)). However, at high H_2O_2 concentrations, inactivation of plant and fungal peroxidases occurs, resulting in the less active compound III and the inactive compound P-670 (25,26). The feedback inhibition by H_2O_2 was also observed in our study (Fig. 1C). The favorable H_2O_2 concentration for MI was 6–14 mM. The range was higher and wider than many other plant peroxidases, such as AOPTP (4–6 mM) (16), SBP (6–10 mM) (15), RPLP (4–6 mM) (18) and SPP (4–7 mM) (17). To meet the requirements of DMI and TTMI, the minimal H_2O_2 concentration should be no less than

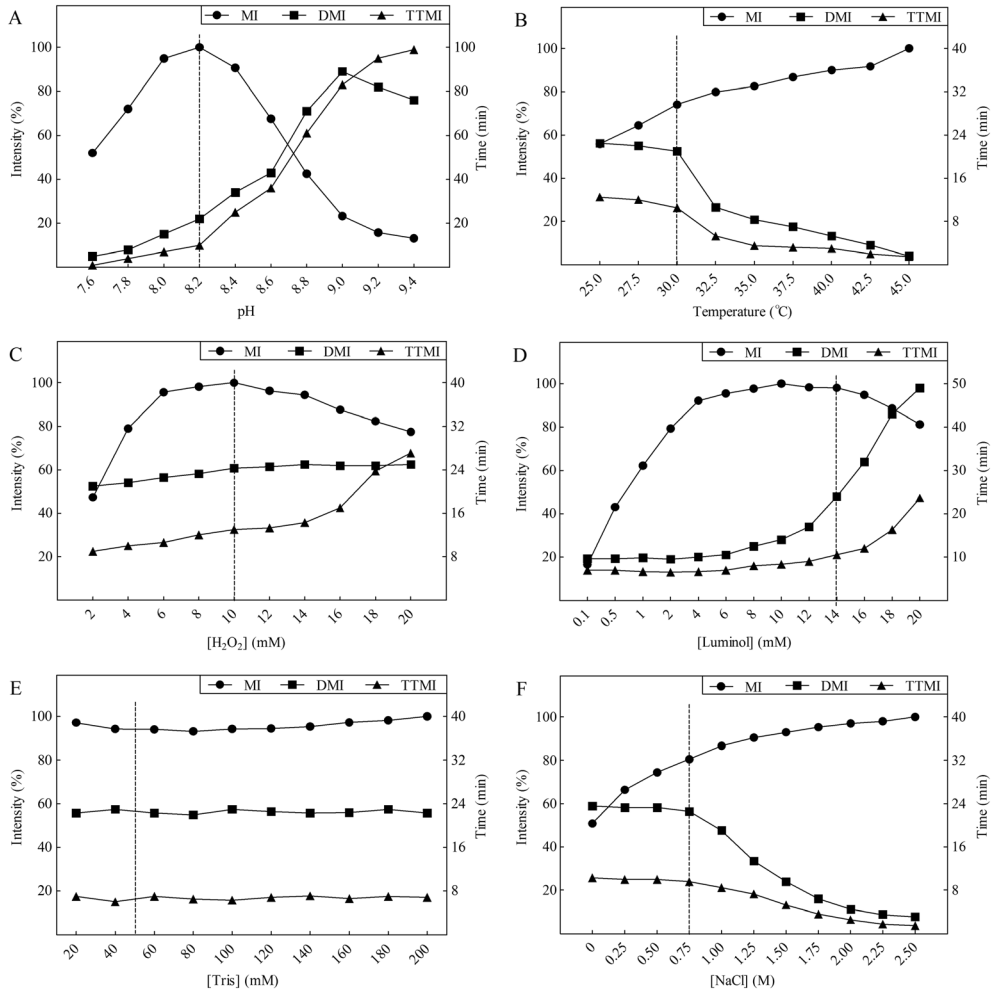

Figure 1. Effect of pH (A), reaction temperature (B) and concentration of H2O2 (C), luminol (D), Tris buffer (E) and NaCl (F) on MI (%, ●), DMI (min, ■) and TTMI (min, ▲) obtained from the luminol–H_2O_2–JcGP1 system. MI, DMI and TTMI indicated the maximum CL intensity, the duration of the maximum CL intensity and the time from initiation of the reaction to the maximum CL intensity, respectively. The highest MI was set as 100%. The optimum point is marked by the dotted line. Conditions: (A) [JcGP1] =100 pM, [H_2O_2] =10 mM, [luminol] =14 mM, [Tris buffer] =50 mM, Temperature =30°C; (B) [JcGP1] =100 pM , [H_2O_2] =10 mM, [luminol] =14 mM, [Tris buffer] =50 mM (pH 8.2); (C) [JcGP1] =100 pM, [luminol] =14 mM, [Tris buffer] =50 mM (pH 8.2), Temperature =30°C; (D) [JcGP1] =100 pM, [H_2O_2] =10 mM, [Tris buffer] =50 mM (pH 8.2), Temperature =30°C; (E) [JcGP1] =100 pM, Tris buffer pH =8.2, [H_2O_2] =10 mM, [luminol] =14 mM, Temperature =30°C; (F) [JcGP1] =100 pM, [H_2O_2] =10 mM, [luminol] =14 mM, [Tris buffer] =50 mM (pH 8.2), Temperature =30°C.

2.0 mM. Within the range 2.0–20 mM, a concentration of 10 mM H_2O_2 was evaluated because MI was the highest at this concentration.

The catalysis properties were also affected by luminol concentration (Fig. 1D). The optimal range of MI was observed from 4–16 mM luminol. A similar correlation pattern was also observed in AOPTP (16), SPP (17) and RPLP (18). The saturated concentration of luminol for JcGP1 catalysis was 5 mM, which was the same as that for SPP (5 mM), but was different from RPLP (9 mM) and AOPTP (14 mM). DMI and TTMI displayed a similar positive correlation pattern with increase in luminol concentration, however the DMI and TTMI began to meet our criteria when the concentration of luminol reached 14 mM. Thus, 14 mM luminol was determined as the optimum concentration.

It has been reported that the catalysis activity of several plant anionic peroxidases for luminol oxidation was highly dependent on Tris buffer concentration of the reaction mixture (15–18). However, in this study, none of the three criteria (MI, DMI and TTMI) was significantly affected by Tris buffer concentration (Fig. 1E). Considering that MI, DMI and TTMI were all strongly affected by pH of the reaction mixer, the 50 mM Tris buffer was used to maintain a more stable pH environment during the reaction.

The optimum NaCl concentration was 0.75 M for luminol oxidation catalysis (Fig. 1F). MI increased with increase in NaCl concentration, whereas DMI and TTMI showed the opposite trend. It has been illustrated that salt concentration was related to JcGP1 activity in the catalysis of guaiacol oxidation (21).

JcGP1 was not sensitive to *p*-iodophenol (a widely used enhancer) at concentration of 0.5–2.0 mM (data not shown), whereas HRP-C showed a high dependence on enhancer in luminol oxidation (10). The ability to catalyze the luminol oxidation efficiently without enhancers was also observed in several other anionic peroxidases (15–18). Recent studies have shown that some of these anionic peroxidases are able to catalyze the luminol reaction to a greater extent in the presence of certain enhancers. For instance, the enhancer 3-(1′-phenothiazinyl)propane-1-sulfonate (SPTZ) with the addition of 4-morpholinopyridin (MORP) could increase the light intensity of SBP- and SPP-induced CL reactions (27,28). Future studies are

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needed to characterize the catalysis properties of JcGP1 enhanced by SPTZ–MORP.

As discussed above, the favorable conditions for the JcGP1-catalyzed oxidation of luminol was 50 mM Tris–HCl buffer (pH 8.2) containing 10 mM H_2O_2, 14 mM luminol and 0.75 M NaCl at 30°C. The DMI and TTMI under these conditions were 22.6 min and 9.6 min, respectively. The concentrations of H_2O_2 and luminol in the study are relatively high compared with previous studies, which can lead to high background and less sensitivity during determination of the JcGP1 concentration. However these conditions were determined based on needs of the present study. Favorable conditions may vary and still need to be optimized in future if the requirements of the CL signal stability and sensitivity vary. The studies listed below were performed under favorable conditions.

Kinetic curves comparison

A comparison of kinetic curves of luminol oxidation catalyzed by HRP-C, SBP and JcGP1 is shown in Fig. 2. The optimum reaction conditions for HRP-C and SBP were performed as reported previously (13,15). The kinetic curve of HRP-C reached its peak shortly after initiation of the reaction and declined rapidly with time in the presence of *p*-iodophenol, whereas the kinetic curves for SBP and JcGP1 remained unchanged long term after reaching their MI.

In the *p*-iodophenol-enhanced CL reaction, HRP-C inactivation could be the main reason for the CL signal decline. The reactive radical species formed during the *p*-iodophenol oxidation have been reported to be the main inactivating agents (13). JcGP1 may also catalyze oxidation of substrates by the 'ping-pong' mechanism as described above. In luminol oxidation catalyzed by JcGP1, the activity of JcGP1 may be less impacted by the accumulation of inactivating agents because the luminol oxidation was induced without any enhancer. Thus, this might elucidate why JcGP1 exhibited a long-term stable CL signal.

The cocktail enhancer SPTZ–MORP was reported to provide a more stable signal for the HRP-C-based enhanced CL reaction (14) and this finding has already been applied in sensitive ELISAs to determine different analytes (29–32). However, JcGP1 may still be attractive because its enhancer-free capability may simplify the development of new ELISA kits.

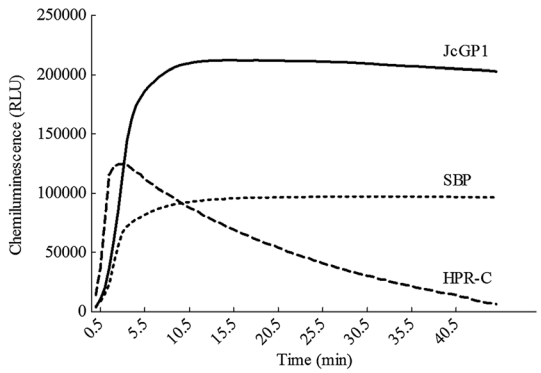

Figure 2. Kinetic curves of CL intensity through luminol oxidation by H_2O_2 in the presence of HRP-C, SBP or JcGP1. Conditions for HRP-C catalysis: [HRP-C] =100 pM, [H_2O_2] =2 mM, [luminol] =1 mM, [*p*-iodophenol] =0.5 mM, [Tris buffer] =50 mM (pH 8.4); Condition for SBP catalysis: [SBP] =100 pM, [H_2O_2] =8 mM, [luminol] =10 mM, [Tris buffer] =100 mM (pH 8.4); Condition for JcGP1 catalysis: [JcGP1] =100 pM, [H_2O_2] =10 mM, [luminol] =14 mM, [NaCl] =0.75 M, [Tris buffer] =50 mM (pH 8.2). CL intensity was recorded for 45 min.

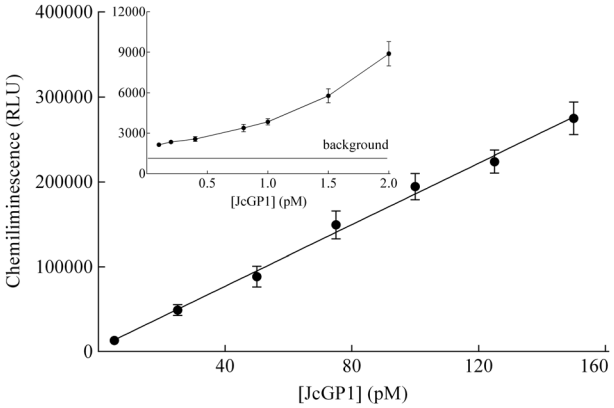

Figure 3. Calibration curve for quantitative analysis of JcGP1 using the reaction of luminol oxidation. Condition: [H_2O_2] =10 mM, [luminol] =14 mM, [NaCl] =0.75 M, [Tris buffer] =50 mM (pH 8.2), Temperature =30°C. CL intensity was collected at 20 min after the initiation of reaction.

Detection limit of JcGP1

The lower detection limit (LDL) of JcGP1 was 0.2 pM under favorable conditions (Fig. 3 insert). The LDL was determined by the 2σ method in this study for comparison with previous studies. In this method, LDL was defined as the concentration of peroxidase, at which the level of CL signal exceeds two-fold than that observed in a peroxidase-free solution. The LDL of JcGP1 was higher compared with SPP (0.01pM) (17), but lower than several anionic peroxidases, 0.3 pM for SBP (15), 2 pM for AOPTP (16) and 1 pM for RPLP (18). At present, the 3σ method has been used to determine the LDL of HRP-C for the SPTZ–MORP-enhanced CL reaction (14). The comparison of LDL calculated by different methods was not addressed here because it might be misleading.

Standard curve and linear interval for JcGP1

The dependence of the CL signal versus the JcGP1 concentration was linear over a broad range of enzyme concentration (10–150 pM) (Fig. 3). This feature was of considerable importance for the use of JcGP1 in quantitative analysis. The best fitting of the experimental data was reached with the linear function Y =1812x + 4628 (r^2 = 0.995, n =6).

Conclusion

We demonstrated that JcGP1 was able to catalyze luminol oxidation in the presence of H_2O_2. Luminol oxidation induced by JcGP1 produced a long-term stable CL signal and exhibited a low detection limit. Compared with HRP-C, the JcGP1-induced reaction was enhancer independent which may make the future ELISAs simpler.

This study demonstrated the optimum point as well as the relationship between the three responses (MI, DMI and TTMI) and the various reaction parameters. The goal of optimization in this study was not only to determine the maximum MI, but also to balance sensitivity (MI), analysis reliability (DMI) and analysis throughput (TTMI). Thus, the optimum reaction conditions for individual practical application may vary according to the different requirements on sensitivity, reliability and throughput.

Previous studies have reported that JcGP1 exhibits long shelf-life, high thermal stability, wide pH resistance and high organic

solvents tolerance, which are important for long-term storage of peroxidase–antibody or peroxidase–antigen conjugates (21). In addition, the simple purification process and the abundance of the *Jatropha curcas* leaves allow the possibility of large scale manufacture of JcGP1. Therefore, the features described above indicate that JcGP1 might be a promising peroxidase candidate for practical application in enzyme immunoassays with chemiluminescent detection.

Acknowledgements

This work was supported by the National Nature Science Foundation of China (Grand Number: 81271178).

References

1. Passardi F, Cosio C, Penel C, Dunand C. Peroxidases have more functions than a swiss army knife. Plant Cell Rep 2005;24:255–65.
2. Hiraga S, Sasaki K, Ito H, Ohashi Y, Matsui H. A large family of class III plant peroxidases. Plant Cell Physiol 2001;42:462–8.
3. Klibanov AM, Morris ED. Horseradish peroxidase for the removal of carcinogenic aromatic amines from water. Enzyme Microb Technol 1981;3:119–22.
4. Wu Y, Taylor KE, Biswas N, Bewtra JK. A model for the protective effect of additives on the activity of horseradish peroxidase in the removal of phenol. Enzyme Microb Technol 1998;22:315–22.
5. Adam W, Lazarus M, Saha-Moller CR, Weichold O, Hoch U, Haring D, et al. Biotransformations with peroxidases. Adv Biochem Eng/Biotechnol 1999;63:73–108.
6. Ferrari RP, Laurenti E, Trotta F. Oxidative 4-dechlorination of 2,4,6-trichlorophenol catalyzed by horseradish peroxidase. J Biol Inorg Chem 1999;4:232–7.
7. Porstmann T, Kiessig ST. Enzyme immunoassay techniques an overview. J Immunol Methods 1992;150:5–21.
8. Roda A, Guardigli M. Analytical chemiluminescence and bioluminescence: latest achievements and new horizons. Anal Bioanal Chem 2012;402:69–76.
9. Zhao L, Sun L, Chu X. Chemiluminescence immunoassay. Trends Anal Chem 2009;28:404–15.
10. Whitehead TP, Thorpe GHG, Carter TJN, Groucutt C, Kricka LJ. Enhanced luminescence procedure for sensitive determination of peroxidase-labelled conjugates in immunoassay. Nature 1983;305:158–9.
11. Lind J, Merenyi G, Eriksen TE. Chemiluminescence mechanism of cyclic hydrazides such as luminol in aqueous solutions. J Am Chem Soc 1983;105:7655–61.
12. Easton PM, Simmonds AC, Rakishev A, Egorov AM, Candeias LP. Quantitative model of the enhancement of peroxidase-induced luminol luminescence. J Am Chem Soc 1996;118:6619–24.
13. Kapeluich YL, Rubtsova M, Egorov AM. Enhanced chemiluminescence reaction applied to the study of horseradish peroxidase stability in the course of *p*-iodophenol oxidation. J Biolumin Chemilumin 1997;12:299–308.
14. Vdovenko MM, Demiyanova AS, Chemleva TA, Sakharov IY. Optimization of horseradish peroxidase-catalyzed enhanced chemiluminescence reaction by full factorial design. Talanta 2012;94:223–6.
15. Alpeeva IS, Sakharov IY. Soybean peroxidase-catalyzed oxidation of luminol by hydrogen peroxide. J Agric Food Chem 2005;53:5784–8.
16. Sakharov IY. Long-term chemiluminescent signal is produced in the course of luminol peroxidation catalyzed by peroxidase isolated from leaves of african oil palm tree. Biochemistry (Mosc) 2001;66:515–9.
17. Alpeeva IS, Sakharov IY. Luminol–hydrogen peroxide chemiluminescence produced by sweet potato peroxidase. Luminescence 2007;22:92–6.
18. Alpeeva IS, Sakharov IY. Luminol oxidation catalyzed by royal palm leaf peroxidase. Appl Biochem Microbiol 2007;43:25–8.
19. Vdovenko MM, Stepanova AS, Eremin SA, Van Cuong N, Uskova NA, Sakharov IY. Quantification of 2,4-dichlorophenoxyacetic acid in oranges and mandarins by chemiluminescent ELISA. Food Chem 2013;141:865–8.
20. Vdovenko MM, Zubkov AV, Kuznetsova GI, Ciana LD, Kuzmina NS, Sakharov IY. Development of ultra-sensitive soybean peroxidase-based CL-ELISA for the determination of human thyroglobulin. J Immunol Methods 2010;362:127–30.
21. Cai F, OuYang C, Duan PP, Gao S, Xu Y, Chen F. Purification and characterization of a novel thermal stable peroxidase from Jatropha curcas leaves. J Mol Catal B: Enzym 2012;77:59–66.
22. Ohlsson PI, Paul KG. Horseradish peroxidase with 2,4-modified haematins, including vinyl homologues. Biochim Biophys Acta 1973;315:293–305.
23. Nissum M, Schiodt CB, Welinder KG. Reactions of soybean peroxidase and hydrogen peroxide pH 2.4–12.0, and veratryl alcohol at pH 2.4. Biochim Biophys Acta 2001;1545:339–48.
24. Kulmacz RJ. Prostaglandin H synthase and hydroperoxides: peroxidase reaction and inactivation kinetics. Arch Biochem Biophys 1986;249:273–85.
25. Baynton KJ, Bewtra JK, Biswas N, Taylor KE. Inactivation of horseradish peroxidase by phenol and hydrogen peroxide: a kinetic investigation. Biochim Biophys Acta 1994;1206:272–8.
26. Valderrama B, Ayala M, Vazquez-Duhalt R. Suicide inactivation of peroxidases and the challenge of engineering more robust enzymes. Chem Biol 2002;9:555–65.
27. Vdovenko MM, Ciana LD, Sakharov IY. 3-(10′-Phenothiazinyl)propane-1-sulfonate is a potent enhancer of soybean peroxidase-induced chemiluminescence. Anal Biochem 2009;392:54–8.
28. Vdovenko MM, Della Ciana L, Sakharov IY. Enhanced chemiluminescence: a sensitive analytical system for detection of sweet potato peroxidase. Biotechnol J 2010;5:886–90.
29. Sakharov IY, Demiyanova AS, Gribas AV, Uskova NA, Efremov EE, Vdovenko MM. 3-(10′-Phenothiazinyl)propionic acid is a potent primary enhancer of peroxidase-induced chemiluminescence and its application in sensitive ELISA of methylglyoxal-modified low density lipoprotein. Talanta 2013;115:414–7.
30. Vdovenko MM, Lu CC, Yu FY, Sakharov IY. Development of ultrasensitive direct chemiluminescent enzyme immunoassay for determination of aflatoxin M1 in milk. Food Chem 2014;158:310–4.
31. Vdovenko MM, Hung CT, Sakharov IY, Yu FY. Determination of okadaic acid in shellfish by using a novel chemiluminescent enzyme-linked immunosorbent assay method. Talanta 2013;116:343–6.
32. Yu FY, Gribas AV, Vdovenko MM, Sakharov IY. Development of ultrasensitive direct chemiluminescent enzyme immunoassay for determination of aflatoxin B1 in food products. Talanta 2013;107:25–9.